Seasonal variation of the sprouting ability of rhizome/root buds and concentrations of storage compounds in *Calystegia sepium* (L.) R. Br. and *Convolvulus arvensis* L.

Jahreszeitliche Veränderung der Austriebsfähigkeit der Rhizom- und Wurzelknospen und der Speicherstoffkonzentrationen von Calystegia sepium (L.) R. Br. und Convolvulus arvensis L.

Leonie Willeke[1]*, Hansjörg Kraehmer[3], Roland Gerhards[1] & Wilhelm Claupein[2]
[1]Institute of Phytomedicine, University of Hohenheim, 70593 Stuttgart, Germany
[2]Institute of Crop Production and Grassland Research, University of Hohenheim, 70599 Stuttgart, Germany
[3]Bayer CropScience; Industrial Park, 65926 Frankfurt am Main, Germany
*Corresponding author, Leonie.Willeke@uni-hohenheim.de

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Summary

Convolvulus arvensis (CONAR) and *Calystegia sepium* (CAGSE) are widespread perennial weeds. Our field results demonstrate that the early application of herbicides usually does not result in complete control. Seasonal changes of the sprouting ability of rhizomes or root buds and the transport of different organic compounds within plants were therefore analyzed as possible indicators for times of maximum phloem and herbicide transport. Rhizome and root pieces from fields in Southern Germany were used. CAGSE buds remained completely dormant until March. Their sprouting ability increased up to over 90 % in May. CONAR buds sprouted only from May onwards and reached a maximum of over 90 % in June. After September, the sprouting ability decreased sharply until the underground organs became dormant in November.

Greenhouse experiments were carried out to analyze the carbon and nitrogen concentration of roots and rhizomes with isotopic ratio mass spectrometer analysis (IRMS), sugars (fructose, glucose, sucrose) by using high-performance liquid chromatography (HPLC) and starch concentrations by polarimeter. Experiments started at the time of first shoots in spring and finished with the onset of winter dormancy in fall. Our results show that CAGSE exhausts starch in rhizomes up to mid-June and CONAR in roots up to late June. Thereafter, the amount of storage compounds increased again. By October, the starch concentration of dry weight in roots and rhizomes reached 11-12 % more than in spring. The November values were 21 % and 18 %, respectively. Sugar concentrations vary during the growing period from 1.5 % to 6 % related to dry weight. At the end of June, an increase was recorded in parallel to starch accumulation. These results are discussed as parameters for the control of both species.

Keywords: Bindweed, carbon, nitrogen, perennial weed, starch, sugar

Zusammenfassung

Convolvulus arvensis (CONAR) und *Calystegia sepium* (CAGSE) sind weit verbreitete mehrjährige Unkräuter. Unsere Feldversuche haben gezeigt, dass eine frühe Herbizidapplikation in der Regel zu keiner vollständigen Bekämpfung führt. Aus diesem Grund wurden die saisonalen Veränderungen der Austriebsfähigkeit von Rhizom- und Wurzelknospen und der Transport verschiedener organischer Verbindungen in den Pflanzen als mögliche Indikatoren für die Zeiten des maximalen Phloem- und Herbizidtransports analysiert. Es wurden Rhizom- und Wurzelstücke von Ackerflächen in Süddeutschland verwendet. Die Rhizomknospen von CAGSE blieben bis März dormant. Ihre Austriebsfähigkeit stieg im Mai auf über 90 %. Die Wurzelknospen von CONAR trieben ab Mai aus und erreichten ein Maximum von über 90 % im Juni. Die Keimfähigkeit fiel ab September stark ab, bis im November die unterirdischen Organe vollständig dormant waren.

Gewächshausversuche zur Bestimmung der Kohlenstoff- und Stickstoffkonzentration in Wurzeln und Rhizomen wurden durchgeführt. Die Analysen erfolgten mit einem Isotopenverhältnis-Massenspektrometer (IRMS), Zuckerkonzentrationen (Glucose, Fructose, Saccharose) wurden mittels Hochleistungs-Flüssigchromatographie (HPLC) gemessen, Stärkekonzentrationen mit dem Polarimeter. Die Experimente begannen mit dem ersten Austrieb im Frühjahr und endeten im Herbst zu Beginn der Winterruhe. Unsere Ergebnisse zeigen, dass die untersuchten CAGSE-Rhizome bis Mitte Juni und CONAR-Wurzeln bis Ende Juni vermehrt Stärke abbauten. Im Anschluss stieg die Speicherstoffkonzentration wieder an. Im Oktober lag die Stärkekonzentration bezogen auf die Trockenmasse in den Wurzeln und Rhizomen um 11-12 % höher als im Frühjahr. Im November lagen die Werte bei 21 % bzw. 18 %. Zuckerkonzentrationen schwankten bezogen auf das Trockengewicht während der Vegetationsperiode zwischen 1,5 % und 6 %. Ende Juni wurde wie bereits bei der Stärke eine Zunahme gemessen. Die Ergebnisse werden als Parameter der Bekämpfung der beiden Arten diskutiert.

Stichwörter: Kohlenstoff, perennierende Unkräuter, Stärke, Stickstoff, Winden, Zucker

1. Introduction

CAGSE and CONAR are perennial weeds, which can twine around crop plants and break down their stems. The two species are distributed worldwide. Especially CONAR is ranked among the most aggressive weeds for years (HOLM et al., 1977). In contrast to the small knowledge about the damage caused by CAGSE, the economic impact of CONAR is well described. According to BOLDT (1998), the estimated loss through bindweed in the USA amounts to over $ 377 million per year (BOLDT et al., 1998). On sites with high weed densities, yield losses were recorded at 50-60 % for CONAR (CALLIHAN et al., 1990). Both species can overwinter after the aboveground parts die in fall. The main and lateral roots become woody and starch and sugar are stored (KOGAN, 1986; WIESE and PHILIPPS, 1976). Dormant rhizome and root buds enable perennial plants in temperate climates to survive even under adverse environmental conditions and to slow or increase the active growth in dependence of the seasonal climatic change (MCALLISTER and HADERLIE, 1985). Without a proper control strategy, the two species increasingly expand their root or rhizome system from year to year. Thus, a CONAR nest can enlarge in one year on average 3 m (FRAZIER, 1943). The formation of extended patches and the high variability of the sprouting time of rhizomes or roots in spring significantly hamper weed control.

CAGSE and CONAR can be found in both conventional and organic farming. Above all, the increasing use of reduced tillage leads to an increase in perennial weeds (NKURUNZIA et al., 2003). In conventional agriculture, specific herbicides therefore have to be used for targeted reduction. The above-ground shoots can easily be killed, but only systemic herbicides can prevent the regrowth of the underground parts (RASK and ANDREASEN, 2007).

In our field experiments, early herbicide application did not result in long-term control of the two bindweeds. Many rhizome and root pieces of the clones formed new shoots in the same or in the next growing season despite herbicide applications. The effective implementation of good control strategies at the right time requires an advanced knowledge about the biology of bindweed.

Studies of NKURUNZIZA (2010) about the perennial species *Cirsium arvense* have already shown that storage substances are used to form new shoots at the beginning of the vegetation period. High photosynthesis rates seem to enhance the carbohydrate transport into rhizomes and roots. Manufacturers, extension service and university programs recommend to apply herbicides to actively-growing bindweeds. Some refer to the enhanced phloem transport. We assume that systemic herbicides are more efficiently translocated into belowground organs when photosynthetic transport is high. Based on this assumption, we examined the sprouting ability of the rhizome and root pieces on the one hand and the concentration variation of stored nutrients like sugars and starch on the other hand as well as the carbon and nitrogen concentration during one growing season.

2. Materials and methods

2.1 Analysis of the sprouting ability of rhizome and root pieces

From March 2010 until the dormancy of rhizome and root pieces in the end of November, pieces with six nodes and a length of about 10 cm were collected each month. The bindweed originated from a field on the Ihinger Hof (Experimental Station of the University of Hohenheim, Germany) and hedge bindweed from a field near Uhingen (Germany). After collection, the underground organs were washed, wrapped in filter paper and placed in a pot with water. The pots were placed in a greenhouse with a day-night cycle of 12 h / 12 h at 23 °C. After seven days, the number of sprouted pieces was noted and documented as a percentage of totally assessed plants.

2.2 Analysis of different ingredients of rhizome and root pieces

At the beginning of the vegetation period (April 2010), vegetatively propagated root and rhizome pieces with 6-7 nodes of field bindweed and hedge bindweed were planted separately in 13 x 13 x

22 cm pots. The plants were grown in a clay-substrate mixture (pH 7.2) and placed in the outside area of a vegetation hall of the University of Hohenheim (Stuttgart, Germany). The growth conditions such as day-night cycle and temperature corresponded to ambient conditions. The plants were additionally watered when necessary. On the first and fifteenth of each month, six plants were collected, the roots freed from soil and the concentration of the ingredients analyzed as described below.

Carbon and nitrogen content were measured by using isotope ratio mass spectrometry (IRMS). For this analysis, the root samples were dried for three hours at 80 °C and mortared in liquid nitrogen to a fine powder. Samples of 2-3 mg were weighed into tin capsules (5 x 9 mm). IRMS was performed on a Thermo Finnigan Delta plus XP system, coupled to a Euro EA elemental analyzer (Euro Vector Instruments and Software, Hekatech, Wegberg, USA) (oxidation furnace, 1000 °C, reduction furnace, 650 °C, carrier gas, 40 kPa; packed column temperature, 90 °C). As standard material, acetanilides were utilized. For data acquisition and processing, Thermo Electron ISO Date NT software, version 2.0, was used.

In preparation of the samples for the polarimetric starch determination by Baumann Grossfeld, 2.5 g of fresh rhizome and root pieces were homogenized with an Ultra-Turrax, hydrochloric acid was added and heated in a water bath at 100 °C. Then a Carrez clarification was conducted to precipitate unwanted substances such as proteins. Finally, the samples were filtered and measured on a Perkin Elmer polarimeter.

For the high-performance liquid chromatography (HPLC) measurement of sugar analysis, 2.5 g of the rhizome and root pieces were first homogenized in a water-acetonitril mixture by using an Ultra-Turrax, then centrifuged and the pellet was discarded. The analysis was performed on a Perkin Elmer HPLC system (evaporative light scattering detector (ELSD), using a HPLC column (ShodexAsahipak NH2P-50, 250 x 4.6 mm, 5 microns), flow rate 1 ml/min; demineralized eluent, acetonitrile and distilled water).

2.3 Statistical analysis

The experiments were conducted in a completely randomized block design. Analysis of variance (ANOVA) was performed on the data from both experiments. The statistical analysis was conducted using SAS 9.2. and the graphics were created with Excel 2007. For the analysis of the root ingredients we used the glm procedure for pairwise comparisons to test for significant variations of different months in vegetation periods on the response variables of carbon, nitrogen or carbohydrates.

3. Results

3.1 Sprouting ability of rhizome and root buds

Figure 1 shows the sprouting ability of the rhizome and root pieces of the two perennial species. The onset of sprouting ability was recorded in April. At this month, the rhizome sprouting ability of CAGSE was 15 % and reached a maximum of 98 % in May. In contrast to the rhizomes, the roots were dormant until May. In June, a large number of pieces (94 %) formed new shoots. The underground organs of both weed species could sprout to a rate of over 78 % throughout the summer until late September. In general, the sprouting ability of the 10 cm long underground organs of field bindweed was on average 10 % higher than that of hedge bindweed. In September, the high sprouting ability decreased significantly and CAGSE pieces became dormant in November and CONAR pieces in December.

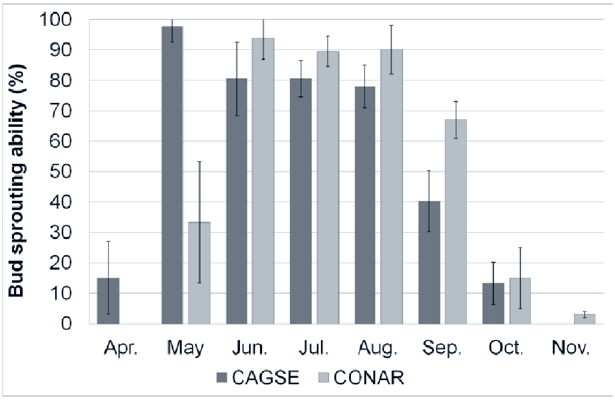

Fig. 1 Bud sprouting ability (%) of rhizome pieces of CAGSE and root pieces of CONAR from April to November with standard deviation.

Abb. 1 *Knospenaustrieb (%) der CAGSE-Rhizomstücke und CONAR-Wurzelstücke von April bis November mit Standardabweichung.*

3.2 Analysis of carbon content

The percentage of total carbon content of the underground organs of both species showed a similar trend (Fig. 2). At the beginning of the growing season, the carbon content of CAGSE and CONAR pieces was 34 % relative to total dry weight. In mid-May, during the begin of shoot growth, carbon content was reduced to 27 %. While the carbon content of CAGSE rhizomes was reduced again in mid-June, the carbon of CONAR rised slowly. After a significantly higher local maximum of about 35 mg of carbon per gram of root or rhizome in mid-July, the content of both weeds obtained until mid-August (that means at the end of flowering until the end of the growing season) showed a nearly constant maximum of approximately 42 %.

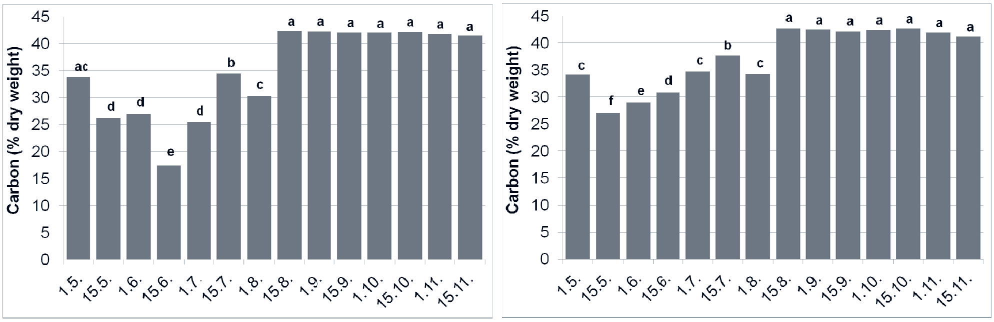

Fig. 2 Carbon content (% dry weight) in the rhizomes of CAGSE (left panel) and roots of CONAR (right panel) from early May until mid-November. Values indicated by different letters are significantly different (P < 0.05).

Abb. 2 *Kohlenstoffkonzentration (% Trockengewicht) der CAGSE-Rhizome (linke Graphik) und CONAR-Wurzeln (rechte Graphik) von Anfang Mai bis Mitte November. Mit unterschiedlichen Buchstaben gekennzeichnete Balken unterscheiden sich signifikant (P < 0,05).*

3.3 Analysis of nitrogen content

The nitrogen content of the underground organs of the bindweeds increased at the beginning of the growing season and was highest from mid-May to early July. The values were about 2 % of dry mass. In addition, Figure 3 shows that the content decreased rapidly in the coming months. In early August at the flowering period, the lowest concentration of less than 1 % was measured. From the end of flowering, in mid-August, the nitrogen content increased again. By the end of the growing season, it changed only slightly and the contents no longer differed significantly.

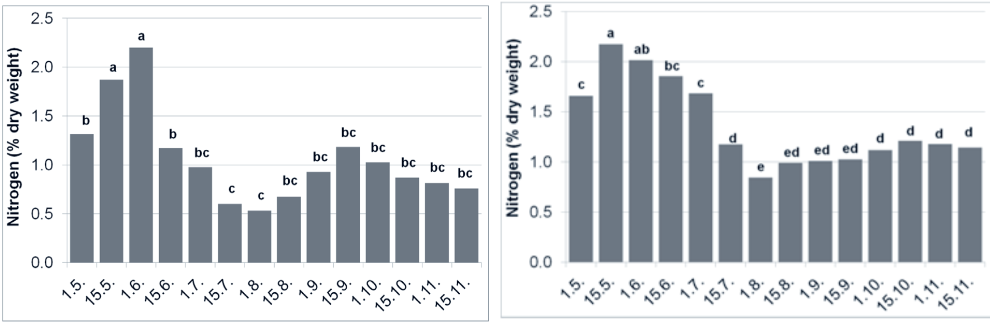

Fig. 3 Nitrogen content (% dry weight) in the rhizomes of CAGSE (left panel) and roots of CONAR (right panel) from early May until mid-November. Values indicated by different letters are significantly different (P < 0.05).

Abb. 3 *Stickstoffkonzentration (% Trockengewicht) der CAGSE-Rhizome (linke Graphik) und CONAR-Wurzeln (rechte Graphik) von Anfang Mai bis Mitte November. Mit unterschiedlichen Buchstaben gekennzeichnete Balken unterscheiden sich signifikant (P < 0,05).*

3.5 Analysis of starch content

Figure 4 shows that, in both species, the starch concentration in the rhizomes and roots was highest at the beginning of planting time compared to the other months. The starch content of CONAR roots increased slowly to a minimum of 12 % at 15.06. The CAGSE rhizomes possessed the lowest starch concentrations of 9-13 % by mid-July. These differed significantly from the levels measured at other times.

An increased storage of starch occured in both species at early to mid-July. From the end of the flowering period, the content varied only slightly and no longer changed significantly. In general, the figure shows that CAGSE as well as CONAR stored about 20 % starch as a reserve.

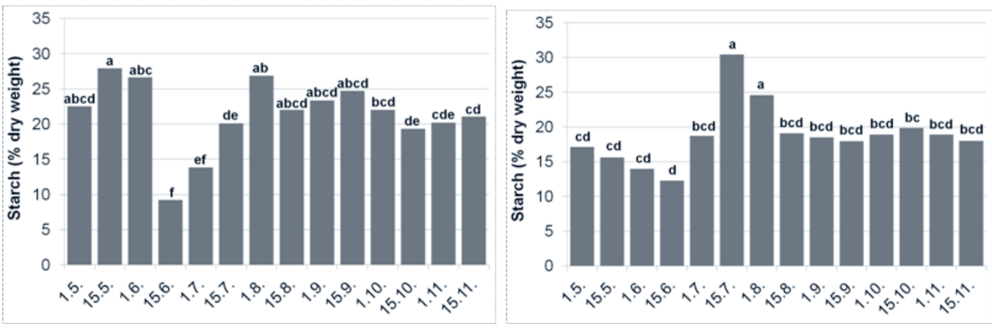

Fig. 4 Starch content (% dry weight) in the rhizomes of CAGSE (left panel) and roots of CONAR (right panel) from early May until mid-November. Values indicated by different letters are significantly different (P < 0.05).

Abb. 4 Stärkekonzentration (% Trockengewicht) der CAGSE-Rhizome (linke Graphik) und CONAR-Wurzeln (rechte Graphik) von Anfang Mai bis Mitte November. Mit unterschiedlichen Buchstaben gekennzeichnete Balken unterscheiden sich signifikant (P < 0,05).

3.5 Analysis of sugar content

The sugar concentrations of both species strongly changed during the vegetation period. In the early stages of growth, especially the glucose level raised in the rhizome and root pieces from approximately 0.5 and 0.8 % to 4 % and 2 %. Figure 5 illustrates that at the time of flowering and seed production the total sugar concentration of the underground organs of both weeds decreased significantly. In mid-August, the lowest concentrations of approximately 1.5 % were measured. From September onwards, sugar was increasingly stored - at this time, in both species, almost exclusively in the form of sucrose.

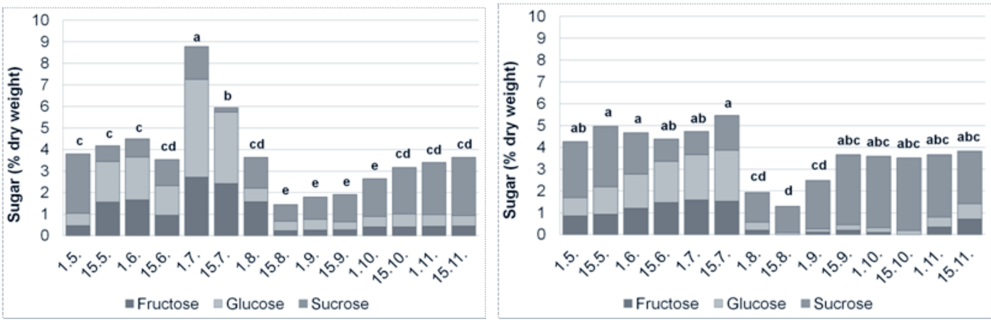

Fig. 5 Sugar content (% dry weight) in the rhizomes of CAGSE (left panel) and roots of CONAR (right panel) from early May until mid-November. Values indicated by different letters are significantly different ($P < 0.05$).

Abb. 5 *Zuckerkonzentration (% Trockengewicht) der CAGSE-Rhizome (linke Graphik) und CONAR-Wurzeln (rechte Graphik) von Anfang Mai bis Mitte November. Mit unterschiedlichen Buchstaben gekennzeichnete Balken unterscheiden sich signifikant (P < 0,05).*

4. Discussion

The two studies show the percentage of sprouting ability of rhizomes and root pieces on the one hand and the trend of ingredient concentrations of the underground storage organs of the two species CAGSE and CONAR on the other hand. Although in the first experiment the root and rhizome pieces were collected from the same nests (which could suggest a possible cloning), there was a high variability in the sprouting ability in the respective months. The sprouting ability was highest from May (CAGSE) and June (CONAR) to August. But never did 100 % of the rhizomes or roots sprout. This high variability of sprouting time during the growing season seems to be a major survival strategy of the plants after herbicide application. This applies especially to early applications before the buds of the rhizome and root pieces sprout and thus the herbicide cannot adequately control the underground organs. In addition to the inhomogeneous sprouting in the second experiment, an inhomogeneous shoot growth of the different plants of one replication was found.

At the beginning of the growth period the stored nutrients were mobilized. The starch granules were presumably reduced and transported as an energy source into the developing stem (sink). The resulting decrease of the starch in underground parts until mid-June is illustrated in Figure 5. Only through increased photosynthesis with increasing shoot growth in June, the source and the sink might have reversed after reaching their compensation point. Assimilates were now transported into the roots and stored in form of sugars and starch. The described transport directions of starch are indicated by measured sugar concentrations. As Figure 4 shows, the glucose level rised sharply, especially in CAGSE rhizomes from mid-June to early July. This could be due to the fact that starch cannot be transported through the phloem, the plants have to convert the starch with the help of phosphorylases and amylases into glucose. In addition, from August onwards, the sucrose concentration increased sharply in the underground parts of both weeds. This is consistent with the theory that the transport of starch in other parts of plants takes place mostly in the form of sucrose

(HELDT, 2003).

The measured carbohydrates and the total carbon and nitrogen analyses underline the seasonal trend of source and sink relationships. In addition to hydrogen and oxygen, carbon is one of the main components of organic compounds in plants (CAMPBELL, 1997) and is very versatile. At the beginning of the growing season, multiple carbon compounds are required as an energy source for growth. Thus the concentration declined first, as can be seen in Figure 2. Presumably only through growth-increased photosynthetic activity with CO_2 fixation in combination with basipetally increased transport of various carbon compounds, did the carbon concentration increase again in August. Also, nitrogen is an important component in plants. It occurs mainly in nucleic acids, proteins, hormones and coenzymes (CAMPBELL, 1997). Proteins also serve alongside carbohydrates and fats as an energy source and serve as fuels of respiration. This could be a reason for the measured decrease in nitrogen content in the underground organs during the shoot growth and the increase only by mid-August (Fig. 3).

Studies that expand knowledge about the biology of perennial species and especially on the sources and sinks of carbohydrate dynamics promise to improve weed management strategies. Compensation point is a pivotal time because it determines the start of increased weed-crop competition (NKURUNZIA, 2010). The right time of herbicide application is very important. Growth stages of the plants, soil moisture and precipitation play an important role (WESTRA, 1992). In recent years, various authors have given recommendations for the optimal time of herbicide treatment. Several studies indicate that herbicide treatments for herbicides such as glyphosate and 2,4-D should take place during the first flowering, when carbohydrate concentration is low (ALCOCK and DICKINSON, 1974; CALLIHAN et al., 1990; KOGAN, 1986; PETERSON, 1998). However, carbohydrate concentrations were not measured in these experiments. It may well be that by observing the actual trend of reserve concentrations, application timing can be optimized. From our studies we can conclude that the compensation point occurs probably before flowering. The starch concentration has reached its minimum 1.5 months before flowering begins. Thus it can be assumed that optimal treatment should take place shortly after the compensation point when the plant has little reserves and when the translocation of the herbicides through the phloem with assimilates into the underground organs can be expected. In addition to the application of herbicides, repeated tillage during the growing season can reduce weed infestation. The formation of new shoots after harrowing cuts depends on the reserves (HAKANSSON, 2003). Thus, in this case, the compensation point is an important time for mechanical control. The reserves of rhizomes and roots can quickly be removed with this strategy.

It should be noted, however, that data from field trials can differ significantly in comparison to greenhouse experiments. Unlike annual weeds, which are grown from seed and thus demonstrate a clearly defined development, perennial species often show large discrepancies as a result of nest-building with extensive underground systems and high phenotypic variability. Therefore, it is necessary to perform further field studies under different environmental conditions. To optimize control strategies, it is important to find out the influence of biotic and abiotic factors on the dormancy of rhizomes and roots, which also affect the seasonal trend of source-sink relations of the reserves.

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